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I further certify that the above application is now proceeding in the name of COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION pursuant to the provisions of Section 113 of the Patents Act 1990.

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Twelfth day of October 2004

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AUSTRALIA
Patents Act 1990

PROVISIONAL SPECIFICATION

200390 Filed 1st October 2003

Invention Title : **Probiotic Storage and Delivery**

Applicant: **Australian Food Industry Science Centre and Commonwealth Scientific & Industrial Research Organisation**

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The invention is described in the following statement:

Probiotic Storage and Delivery

This invention relates to the storage and delivery of probiotic materials to selected sites in the gastrointestinal system.

Background to the invention

Probiotics are live microbial food ingredients that have a scientifically documented beneficial effect on human health. Probiotics are also used in animal feedstuffs either to improve animal health or productivitiy. They are used in pet foods, mainly to decrease unpleasant odours and improve consistency of faecal material.

Most of the dominant global strains of commercial probiotic bacteria belong to the bifidobacteria and the lactobacilli. However, bacteria from other genera are used in some parts of the world. For example, China uses a number of other genera, including *Bacillus* and a *Clostridium*. *Enterococcus faecium* has also been used worldwide, however, this genus is implicated in transfer of antibiotic resistance issues. In the Western world, both bifidobacteria and lactobacilli have a strong track record as safe and acceptable genera to use as probiotics. Other examples are discussed in :

Mogensen, G., Salminen, S., O'Brien, J., Ouwehand, A., Holzapfel, W., Shortt, C., Fonden, R., Miller, GD., Donohue, D., Playne, M., Crittenden, R.,. Bianchi-Salvadori, B.and R. Zink (2002). Food microorganisms – health benefits, safety evaluation and strains with documented history of use in foods *Internat, Dairy Federation Bulletin No:* 377 : 4-9 and Mogensen, G., Salminen, S., O'Brien, J., Ouwehand, A., Holzapfel, W., Shortt, C., Fonden, R., Miller, GD., Donohue, D., Playne, M., Crittenden, R.,. Bianchi-Salvadori, B.and R. Zink (2002) Inventory of microorganisms with a documented history of use in food *Internat, Dairy Federation Bulletin No:* 377: 10-19.

It has been widely recognized by researchers and medical investigators that most health effects are conferred by a specific strain, and mostly not by the species in general. While many research groups have selected strains for useful probiotic properties for manufacture, for incorporation into foods, for survival in the gut, and for health properties, there is a dearth of information on performance in humans published in peer-reviewed journals.

Evidence is increasing that a probiotic food should contain selected strains of both lactobacilli and bifidobacteria. The concept is that the probiotic lactobacilli are useful in the young (where the gut microflora of infants is already naturally rich in bifidobacteria), and that addition of probiotic bifidobacteria becomes more important in the elderly. The numbers of indigenous bifidobacteria decline with ageing if probiotics are not used. Bifidobacteria obviously provide some protection against pathogens which are not able to be done effectively by lactobacilli alone. Adequate viable numbers of the strain of probiotic bacteria in the appropriate segment of the gut are essential if they are to be effective in an health sense. Most authorities consider 10 million bacteria per gram of food an appropriate dietary dose. Technically, this can be quite readily achieved. However, dose response curves have not been produced for any probiotic strain against any health condition.

Losses of bacterial numbers occur during manufacture, freeze drying and during shelf life. However, further losses occur during transit through the gastro-intestinal tract. The probiotic cultures will encounter gastric juices in the stomach ranging from pH 2.5 (on an empty stomach) through to pH 5.0 . The cultures will be resident in the stomach for between 40 minutes and 5 hours. They will also encounter in the stomach and the small intestine,bile salts,lipolytic, hydrolytic and proteolytic enzymes, which are also able to kill bacteria. It is not until the probiotic bacteria reach higher pH regions of the gastro-intestine that they are able to grow or survive. Such regions are the ileum and the bowel. During this transit, the bacteria also have to compete with resident bacteria for space and for nutrients. They also have to avoid being flushed out of the tract by normal peristaltic action, and they have to avoid being killed by anti-microbials produced by other organisms. The bacteria have their most favourable growing conditions in the first third of the large bowel (the proximal bowel).

Ability to adhere to surfaces, such as intestinal mucosal layer, and the epithelial cell walls of the gut are thus important characteristics for a probiotic. The term "colonisation" is used , and means that the bacteria has mechanisms which enable it to survive in a region of the gastro-intestine on an on-going basis. It is generally believed that the microflora of the gastro-intestine are relatively stable in adults, and are not easily altered by changes in the conditions in the gut ecosystem.

Exceptions to this are administration of antibiotics, but even then the gut flora usually re-establish after sometime with a similar species composition.

Mechanisms of Action of probiotic bacteria include:

- competitive exclusion (occupation of niches in the gut mucosal surface to prevent colonisation by infective species)
- production of acid conditions (lactic acid fermentation by the bacteria leading to lowered gut pH)
- effects on immune-mediated response
- reduction of putrefactive and genotoxic intestinal reactions (leading to lower pre-carcinogen levels)
- release of anti-microbials, such as bacteriocins

Many diarrhoeal diseases originate from dysfunction in the small intestine, yet probiotic bacteria are not usually found in high numbers in that region, with the exception of some lactobacilli. There is little direct evidence available from healthy humans on microbial composition of the small intestine region. However, the effectiveness of probiotic bacteria in reducing diarrhoeal disease is quite well established. They are either functioning in transit through the small intestine, or acting through an immune effect. Most immune reactions will occur in the mucosal walls of the small intestine and not the large bowel, thus, if immune-modulation is believed to be the mechanism of action, then the probiotic must be present in the small intestine. The other region of diarrhoeal disturbance is the large bowel. Obviously, probiotic bacteria can establish in that region quite easily.

In addition to diarrhoeal diseases, probiotic bacteria are effective in lessening lactose intolerance, provided bacteria are chosen which have high beta galactosidase enzyme function. Lactose intolerance effects occur in the bowel. There are a large number of other emerging health claims made for probiotics. These centre particularly around the bowel eg., bowel cancer, irritable bowel syndrome and inflammatory bowel diseases (such as Crohn's disease).. Accordingly , release of probiotic lactobacilli in the last half of the small intestine is preferred. Release of bifidobacteria is usually aimed to occur in the large bowel. Greater immune responses tend to occur with bifidobacteria than with lactobacilli, thus, there is an argument that bifidobacteria in the small intestinal regions are of great importance.

Daily consumption of the probiotic is necessary if the target site is in the small intestine, as it is unlikely that the bacteria can adhere to the gut wall in sufficient numbers (except perhaps some lactobacilli). However, daily consumption maynot be necessary if the target site is the large bowel, as growth of the bacteria and colonisation may occur.

Probiotic bacteria with good characteristics for effectiveness against disease and other conditions may not have good survival characteristics (eg resistance to low pH, bile salts, proteolytic and hydrolytic enzymes, resistance to antibiotics, adherence to cell walls). Protection of the bacteria during transit to the target site is usually necessary.

Protection may be achieved in several ways: encapsulation in a slow release pharmaceutical compound; encapsulation in a gum or in alginate; encapsulation in a resistant starch or in inulin in combination with a gum; protection by incorporation in a food containing resistant starch; or in a dairy food where the proteins and fats may provide some protection.

USA patent 5422121 discloses a coating incorporating a film forming polymer having hydrophilic groups and a polysaccharide decomposable in the colon which is useful in delivering dosages to the colon.

USA patent 5840860 discloses the delivery of short chain fatty acids to the colon by covalently linking them to a carbohydrate carrier.

USA patent 6060050 discloses a combination of a probiotic bacteria such as bifidobacterium with high amylose starch as a carrier which also acts as a growth or maintenance medium in the large bowel or other regions of the gastrointestinal tract.

USA patent application 20030096002 discloses a matrix for use in the controlled release of microorganisms. The matrix is formed of a hydrophobic wax and a release modifying agent selected from polysaccharides ,starch, an algae derivative or a polymer.

USA patent 6413494 discloses a colonic drug delivery vehicle consisting of a polysaccharide such as pectin.

Some probiotics need protection during processing as well as during delivery to the gastro intestinal tract. They may be water or oxygen sensitive and need protection to maintain viability during processing storage and transporting.

European patent 1213347 discloses a method of drying and preserving yeasts and microorganisms by mixing them with a matrix material that absorbs water
It is an object of this invention to provide a means of encapsulating probiotics to protect them from deterioration during processing and storage and enable them to be delivered to specific sites in the gastrointestinal tract.

Brief description of the Invention

To this end the present invention provides probiotic bacteria formulations in which the probiotic microorganism is dispersed

a) in an aqueous suspension of a film forming protein and a carbohydrate.
b) in an oil in water emulsion of a film forming protein and a carbohydrate and a fat, or
c) in an oil which is subsequently dispersed in a film forming protein and a carbohydrate

The suspension or emulsion may be dried to form a powder.
Throughout this specification the term probiotic is intended to include microorganisms such as bacteria or fungi either individually or in combination which exhibit a beneficial effect on human health if delivered alive to the target region of the gut. Examples include bifido bacteria, lactobacilli, saccharomyces, lactococci, streptococci, propionibacteria and any other microorganisms which may be demonstrated to have beneficial effects on the host.
The probiotic may be mixed with a prebiotic material or be part of a symbiotic or symbiotic material.
Throughout this specification the term prebiotic means a substance such as a protein, peptide, or carbohydrates that provide nutrients for the probiotic or assist the probiotic. For example lactoferrin can enhance the growth of desirable bacteria. Usually prebiotics cannot be digested in the upper intestinal tract. Prebiotic carbohydrates include resistant starch, potato starch or high amylose starch such as starplus, modified starches (including carboxylated starches, acetylated, propylated, and butyrylated starches), non-digestible oligosaccharides such as fructo-, gluco-, xylo-, soyabean-, galacto-, milk-, inulin-, arabinoxylans,

arabinogalactans, galactomannans or digestion products of these, but not excluding other oligosaccharides able to exert prebiotic effects.

Throughout this specification the term symbiotic or synbiotic means a combination of a probiotic and a prebiotic which together have a synergistic beneficial effect on human health.

The probiotic bacteria may be dispersed in oil and then emulsified with the aqueous suspension and then dried to produce an encapsulated oil carrying probiotic. This may also be dried to produce a powder. Oil suspended probiotics may be preferred where the probiotic is water sensitive. The oil is preferably an edible oil and the emulsion or the powder obtained by drying the emulsion, is used as a food ingredient, as well as in feed supplements.

The aqueous suspension of the carbohydrate and the film forming protein may be heated either before or after the encapsulation step to react the saccharide and protein components. If the saccharide has reducing sugar groups the heating step will produce maillard reaction products. Heated aqueous suspensions are preferred when the probiotic is oxygen sensitive.

The encapsulants of this invention form stable robust films or matrices which embed the probiotic or form films around the probiotic or the oil droplets.

Any protein useful in encapsulating oils can be used as the protein component of this invention. A carbohydrate is combined with the protein.

The protein is preferably soluble and is preferably stable in the heating range of the Maillard reaction and includes casein, soy and whey proteins, gelatine, egg albumin and hydrolysed proteins with increased free amino acid groups including soy protein hydrolysate. Care needs to be taken in reacting the protein and carbohydrate to ensure that the conditions do not result in gelling or coagulation of the protein, as this will render the protein incapable of forming an effective film.

The preferred protein is a milk protein especially casein or whey protein isolate. Casein is a preferred protein in many applications because of its low cost and its greater resistance to gelling during any heat treatment eg: to form Maillard reaction products. For infant food applications whey proteins are the preferred protein source. The amount of Maillard reaction product in the protein-carbohydrate mixture is an amount sufficient to provide antioxidant activity for the period of the product's shelf life. Preferably the minimum reaction required between the protein

and carbohydrate prior to encapsulation consumes at least 10% of the sugar present. The extent of Maillard reaction product formed can be monitored, for a particular protein/carbohydrate combination, by the degree of colour change that occurs. An alternative measure is to assay the unreacted sugar.

It is not essential that the carbohydrate and protein undergo a maillard reaction to be an effective encapsulant for the probiotic bacteria. In mixing the protein and starch it has been found that microfluidisation of the materials particularly the carbohydrate enhances the effectiveness of the formulation.

A preferred carbohydrate is a sugar with a reducing group preferably selected from the group consisting of monosaccharides (eg: glucose, fructose), disaccharides (eg: maltose, lactose), trisaccharides, oligosaccharides and glucose syrups. Any reducing sugar source may be used including honey. Carbohydrates which do not undergo a maillard reaction with the protein may also be used.

It is within the ambit of this invention to use an oligosaccharide, or a starch including a resistant starch to improve the delivery and growth of the probiotic in the intestine and colon. Some of these materials are usually not digested in the upper intestinal tract and can assist in the growth of the probiotic.

Detailed description of the invention

Preferred embodiments of the invention will be described.

In the drawings

Figure 1 illustrates graphically viable counts of *Bifidobacterium lactis* Bb-12 following release from various microcapsules. (The bifidobacteria were cultured on Reinforced Clostridial Agar) ;

Figure 2 illustrates graphically viable counts of *Lactobacillus acidophilus* La-5 following release from various microcapsules. (The lactobacilli were grown on MRS agar).

Materials

Probiotic bacteria (Bifidobacteria, lactobacilli, or blends of a range of probiotic bacteria)

Proteins (casein, whey protein, soy protein, hydrolysed proteins, etc.)

Cabohydates (glucose, lactose, oligosaccharides, polysaccharides, dried glucose syrup, maltodextrins, native starches, modified starches, resistant starches, pre-processed starches, etc).

Lipids (vegetable and animal oils, di- and tri-glycerides, n3- and n6 oils, etc.)

Method of microcapsule preparation

Method 1: Preparation of microcapsules using reacted or unreacted protein-carbohydrate blends with freeze dried bacteria or concentrate dispersed directly into the mixture

- Prepare a mixture of a protein-carbohydrate solution at 60°C. Heat the mixture at 98°C for 30 min. Cool down to 10°C. Disperse the freeze dried bacteria or concentrate into the reacted solution using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o Temperature of the inlet air T_i inlet air temperature, Temperature of the outlet air T_o).
- Prepare a mixture of a protein-carbohydrate solution at 60°C. Cool down to 10°C. Disperse freeze dried bacteria or concentrate into the solution using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.

Method 2: Preparation of microcapsules using reacted or unreacted protein-carbohydrate blends with freeze dried bacteria dispersed in oil prior to addition into the mixture

- Prepare a mixture of a protein-carbohydrate solution at 60°C. Heat the mixture at 98°C for 30 min. Cool down to 10°C. Disperse freeze dried bacteria in oil. Add the freeze dried bacteria dispersion into the reacted solution using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.
- Prepare a mixture of a protein-carbohydrate solution at 60°C. Cool down to 10°C. Disperse freeze dried bacteria in oil. Add the freeze dried bacteria dispersion into the solution using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.

Method 3: Preparation of microcapsules using reacted or unreacted oil-in-water emulsion containing oil-protein-carbohydrate with freeze dried bacteria dispersed directly into the emulsion.

- Prepare a mixture of a protein-carbohydrate solution at 60°C, add the oil and homogenise the mixture at 250 bar. Heat the homogenised emulsion at 98°C for 30 min. Cool down to 10°C. Disperse freeze dried bacteria into the reacted mixture using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.
- Prepare a mixture of a protein-carbohydrate solution at 60°C, add the oil and homogenise the mixture at 250 bar. Cool down to 10°C. Disperse the freeze dried bacteria into the mixture using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.

Method 4: Preparation of microcapsules using protein-microfluidised starch mixture or a protein-raw starch mixture with freeze dried bacteria dispersed in oil prior to addition into the mixture

- Prepare a 15% w/w protein solution at 60°C. Prepare a 10-20% w/w starch dispersion at 60°C, heat the starch dispersion at 121°C for 60 min, cool down, add remaining water to make up to 10% total solids (if originally prepared at 20%) and microfluidise at 800 bar. Mix the protein solution and microfluidised starch together. Cool down to 10°C. Disperse the freeze dried bacteria in oil. Add the freeze dried bacteria dispersion into the protein-starch mixture using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.
- Prepare a 15% w/w protein solution at 60°C. Prepare a 10% w/w starch dispersion at 60°C. Mix the protein solution and starch dispersion together. Cool down to 10°C. Disperse the freeze dried bacteria in oil. Add the freeze dried bacteria dispersion into the protein- starch mixture using a mixer. Spray dry at 120-160°C T_i and / 50-70°C T_o.

Preparation of microcapsules

Example 1 Microencapsulation of freeze dried probiotic bifido bacteria with protein-carbohydrate blend.

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Cool down to 10°C. Disperse bifido bacteria Bb12 (freeze dried powder) into the solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	20%	5.9%	25
Cas-Oligo-DGS Blend	80%	23.5%	100
Water		70.6%	300
Total	100%	100.0%	425

Example 2 Microencapsulation of freeze dried probiotic bifido bacteria in oil with a protein carbohydrate blend.

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Cool down to 10°C. Disperse bifido bacteria Bb12 (freeze dried powder) in oil. Add the bifido bacteria Bb12 dispersion into the solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	10%	3.0%	25
Canola oil	40%	12.0%	100
Cas-Oligo-DGS Blend	50%	15.0%	125
Water		70.0%	583.3
Total	100%	100.0%	833.3

Example 3 Microencapsulation of freeze dried probiotic bifido bacteria in emulsion containing oil and a protein-carbohydrate blend

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C, add oil using a mixer and homogenise at 250 bar. Cool down to 10°C. Disperse bifido bacteria Bb12 powder into the mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	20%	5.9%	25
Cas-Oligo-DGS Blend	72%	21.2%	90
Water		70.6%	300
Oil	8%	2.3%	10
Total	100%	100.0%	425

Example 4 Microencapsulation of probiotic bifido bacteria in oil with protein and high amylose starch

Processing steps

Prepare a 15% w/w sodium caseinate solution at 60°C. Prepare a 10% w/w Hylon VII dispersion at 60°C. Mix the sodium caseinate solution and Hylon VII dispersion together. Cool down to 10°C. Disperse bifido bacteria Bb12 (freeze dried powder) in oil. Add the bifido bacteria Bb12 dispersion into the protein-starch mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	5.0%	0.8%	25
Canola oil	20.0%	3.1%	100
Na Caseinate	37.5%	5.8%	187.5
Water		32.7%	1062.5
Hylon VII	37.5%	5.8%	187.5
Water		51.8%	1682.5
Total	100.0%	100.0%	3245.0

Example 5 Microencapsulation of probiotic bifido bacteria in a heated protein-carbohydrate matrix

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Heat mixture at 98°C for 30 min Cool down to 10°C. Disperse bifido bacteria Bb12 (freeze dried powder) into the reacted solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)

Bifido bacteria Bb12 (Freeze dried)	20%	5.9%	25
Heat processed Cas-Oligo-DGS Blend	80%	23.5%	100
Water		70.6%	300
Total	100%	100.0%	425

Example 6 Microencapsulation of probiotic bifido bacteria in oil with a heat processed protein-carbohydrate mixture

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Heat mixture at 98°C for 30 min. Cool down to 10°C. Disperse the bifido bacteria Bb12 (freeze dried powder) in oil. Add the bifido bacteria Bb12 dispersion into the reacted solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	10%	3.0%	25
Canola oil	40%	12.0%	100
Heat processed Cas-Oligo-DGS Blend	50%	15.0%	125
Water		70.0%	583.3
Total	100%	100.0%	833.3

Example 7 Microencapsulation of probiotic bifido bacteria in an emulsion containing oil and a heat processed protein-carbohydrate blend

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C, add the oil and homogenise at 250 bar. Heat the emulsion at 98°C for 30 min. Cool down to 10°C. Disperse bifido bacteria Bb12 freeze dried powder into the reacted mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	20%	5.9%	25
Cas-Oligo-DGS Blend	72%	21.2%	90
Water		70.6%	300
Oil	8%	2.3%	10
Total	100%	100.0%	425

Example 8 Microencapsulation of probiotic bifido bacteria in oil with protein and a microfluidised high amylose starch

Processing steps

Prepare 15% w/w caseinate solution at 60°C. Prepare 20% w/w Hylon VII dispersion at 60°C, heat 121°C for 60 min, cool down, and add remaining water to make up to 10% w/w total solids and microfluidise at 800 bar. Mix the sodium caseinate solution and microfluidised Hylon VII together. Cool down to 10°C. Disperse bifido bacteria Bb12 powder in oil. Add the bifido bacteria Bb12 dispersion into the protein-starch mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Bifido bacteria Bb12 (Freeze dried)	5.0%	0.8%	25
Canola oil	20.0%	3.1%	100
NaCaseinate	37.5%	5.8%	187.5
Water		32.7%	1062.5
Hylon VII	37.5%	5.8%	187.5
Water		51.8%	1682.5
Total	100.0%	100.0%	833.3

Example 9 Microencapsulation of freeze dried probiotic lactobacilli with a protein-carbohydrate blend.

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Cool down to 10°C. Disperse lactobacilli La-5 (freeze dried powder) into the solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)	
Lactobacilli La-5 (Freeze dried)	20%	5.9%		25
Cas-Oligo-DGS Blend	80%	23.5%		100
Water		70.6%		300
Total	100%	100.0%		425

Example 10 Microencapsulation of freeze dried probiotic lactobacilli in oil with a protein carbohydrate blend.

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Cool down to 10°C. Disperse the lactobacilli La-5 (freeze dried powder) in oil. Add the lactobacilli La-5 dispersion into the solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)	
Lactobacilli La-5 (Freeze dried)	10%	3.0%		25
Canola oil	40%	12.0%		100
Cas-Oligo-DGS Blend	50%	15.0%		125
Water		70.0%		583.3
Total	100%	100.0%		833.3

Example 11 Microencapsulation of freeze dried probiotic lactobacilli in an emulsion containing oil and a protein-carbohydrate blend

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C, add oil using a mixer and homogenise at 250 bar. Cool down to 10°C. Disperse lactobacilli La-5 powder into the mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)	
Lactobacilli La-5 (Freeze dried)	20%	5.9%		25
Cas-Oligo-DGS Blend	72%	21.2%		90
Water		70.6%		300
Oil	8%	2.3%		10
Total	100%	100.0%		425

Example 12 Microencapsulation of probiotic lactobacilli in oil with protein and a high amylose starch

Processing steps

Prepare 15% w/w sodium caseinate solution at 60°C. Prepare 10% w/w Hylon VII dispersion at 60°C. Mix the sodium caseinate solution and Hylon VII dispersion together. Cool down to 10°C. Disperse lactobacilli La-5 (freeze dried powder) in oil. Add the lactobacilli La-5 dispersion into the protein-starch mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Lactobacilli La-5 (Freeze dried)	5.0%	0.8%	25
Canola oil	20.0%	3.1%	100
Na Caseinate	37.5%	5.8%	187.5
Water		32.7%	1062.5
Hylon VII	37.5%	5.8%	187.5
Water		51.8%	1682.5
Total	100.0%	100.0%	3245.0

Example 13 Microencapsulation of probiotic lactobacilli in a heated protein-carbohydrate matrix

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Heat mixture at 98°Cfor 30 min Cool down to 10°C. Disperse lactobacilli La-5 (freeze dried powder) into the reacted solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Lactobacilli La-5 (Freeze dried)	20%	5.9%	25
Heat processed Cas-Oligo-DGS Blend	80%	23.5%	100
Water		70.6%	300
Total	100%	100.0%	425

Example 14 Microencapsulation of probiotic lactobacilli in oil with a heat processed protein-carbohydrate mixture

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C. Heat mixture at 98°C for 30 min. Cool down to 10°C. Disperse lactobacilli La-5 (freeze dried powder) in oil. Add the lactobacilli La-5 dispersion into the reacted solution using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Lactobacilli LA-5 (Freeze dried)	10%	3.0%	25
Canola oil	40%	12.0%	100
Heat processed Cas-Oligo-DGS Blend	50%	15.0%	125
Water		70.0%	583.3
Total	100%	100.0%	833.3

Example 15 Microencapsulation of probiotic lactobacilli in an emulsion containing oil and heat processed protein-carbohydrate

Processing steps

Prepare a mixture containing sodium caseinate, oligosaccharide and dried glucose syrup (Cas-oligo-DGS) solution at 60°C, add oil and homogenise at 250 bar. Heat the emulsion at 98°C for 30 min. Cool down to 10°C. Disperse lactobacilli La-5 freeze dried powder into the reacted mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Lactobacilli La-5 (Freeze dried)	20%	5.9%	25
Cas-Oligo-DGS Blend	72%	21.2%	90
Water		70.6%	300
Oil	8%	2.3%	10
Total	100%	100.0%	425

Example 16 Microencapsulation of probiotic lactobacilli in oil with protein and a microfludised high amylose starch

Processing steps

Prepare 15% w/w caseinate solution at 60°C. Prepare 20% w/w Hylon VII dispersion at 60°C, heat 121°C for 60 min, cool down, and add remaining water to make up to 10% w/w total solids and microfluidise at 800 bar. Mix the sodium caseinate solution and microfluidised Hylon VII together. Cool down to 10°C. Disperse lactobacilli La-5 powder in oil. Add the lactobacilli La-5 dispersion into the protein-starch mixture using a mixer. Spray dry at 160/65°C T_i/T_o.

Ingredient	% dry basis	% wet basis	Wt of ingredient (g)
Lactobacilli La-5 (Freeze dried)	5.0%	0.8%	25
Canola oil	20.0%	3.1%	100
NaCaseinate	37.5%	5.8%	187.5
Water		32.7%	1062.5
Hylon VII	37.5%	5.8%	187.5
Water		51.8%	1682.5
Total	100.0%	100.0%	833.3

Those skilled in the art will realize that this invention may be realized in embodiments differing from those described without departing from the basic teachings of the invention.

CLAIMS

2. An encapsulated probiotic microorganism in which the probiotic microorganism is dispersed
   a) in an aqueous suspension of a film forming protein and a carbohydrate.
   b) in an oil in water emulsion of a film forming protein and a carbohydrate and a fat, or
   c) in an oil which is subsequently dispersed in a film forming protein and a carbohydrate

2. An encapsulated probiotic microorganism as claimed in claim 1 in which the carbohydrate contains a reducing sugar group.

3. An encapsulated probiotic microorganism as claimed in claim 1 or 2 in which a prebiotic material is mixed with the probiotic microorganism.

4. An encapsulated probiotic microorganism as claimed in claim 1or 2 in which the carbohydrate in the film forming composition is a prebiotic carbohydrate.

5. An encapsulated probiotic microorganism as claimed in claim 1 in which the protein is casein or whey protein.

6. An encapsulated probiotic microorganism as claimed in claim 1 or 2 in which the carbohydrate is a resistant starch or a high amylase starch.

7. A probiotic bacteria formulation where the protein and carbohydrate in claim 1 is heat processed

8. A probiotic bacteria formulation where the protein and carbohydrate in claim 1 is heat processed in the presence of an oil or fat prior to addition of the probiotic bacteria.

9. A probiotic bacteria formulation in claim 3 where the starch is processed by heating and/or microfluidisation.

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ABSTRACT

Probiotic microorganisms are micro encapsulated by dispersing the probiotic microorganism is dispersed

a) in an aqueous suspension of a film forming protein and a carbohydrate.
b) in an oil in water emulsion of a film forming protein and a carbohydrate and a fat, or
c) in an oil which is subsequently dispersed in a film forming protein and a carbohydrate

. The emulsion or suspension may be dried to form a powder.

The probiotic may be dispersed in oil and then emulsified with the aqueous suspension and then dried to produce an encapsulated oil carrying probiotic. This may also be dried to produce a powder. Oil suspended probiotics may be preferred where the probiotic is water sensitive. The preferred protein is casein or whey protein and the carbohydrate may be a resistant starch or a saccharide with a reducing sugar group. Where the probiotic is oxygen sensitive the protein carbohydrate is heated to create Maillard reaction products in the encapsulating film.